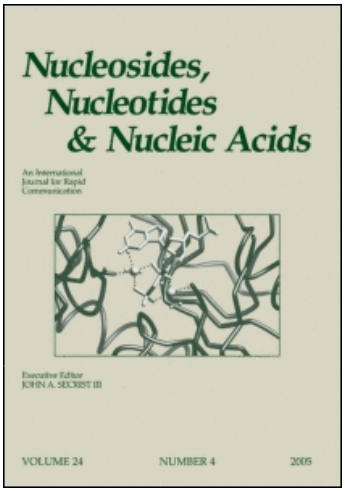

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Rapid Ahalysis of Pseudouridine in Serum of Patients with Chronic Renal Failure, Using Phenylboronic Acid (PBA) Solid-Phase Extraction and HPLC

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RAPID ANALYSIS OF PSEUDOURIDINE IN SERUM OF PATIENTS WITH CHRONIC RENAL FAILURE, USING PHENYLBORONIC ACID (PBA) SOLID-PHASE EXTRACTION AND HPLC.

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Abstract. The rare nucleoside pseudouridine (PSI) was analyzed in sera of endstage renal patients by isocratic HPLC after solid-phase extraction with phenylboronic acid cartridges.

Introduction. Serum concentrations of PSI are elevated in patients with chronic renal failure [1]. Average PSI concentrations in sera of patients treated by CAPD (continuous ambulatory peritoneal dialysis) have been found to be significantly higher as compared to those on regular hemodialysis (94.9 μmol/l versus 55.9 μmol/l respectively). The reverse was observed for an as-yet unidentified fluorescent compound (denoted UKF3), of which the serum concentration is significantly correlated with that of PSI ($r=0.79$, $P<0.0005$; $r=0.67$, $P<0.01$, in hemodialysis and CAPD respectively) [2]. It was found that PSI is not significantly bound to serum protein [2]. Three possible causes have been proposed for the high PSI concentrations in CAPD:
1) Low peritoneal permeability of PSI. 2) Specific t-RNA turnover (related to protein synthesis). 3) Asymptomatic peritonitis accompanied by cell-death, or another as-yet-unrecognized cellular process [2].

In order to facilitate the study of the unexpected behavior of PSI in dialysis treatment, we optimized a rapid HPLC-analysis for this compound.

Results. Serum samples were subjected to solid-phase extraction on Bond-Elut^{R} silica-bound phenylboronic acid (PBA) cartridges (Analytichem, Harbor City, CA90710, USA), using a Vac-Elut^{TM} sample station. We optimized the procedure resulting in the following sequence:

a) wet with 1 ml methanol and 1 ml water;
b) load cartridge with 250 μl serum (buffered to pH 8.6);
c) wash with 350 μl ammoniumacetaat (0.25 mol/l; pH=8.6);
d) elute with 1 ml formic acid (0.1 mol/l);
e) inject eluate directly on HPLC; (isocratic 95/5 v/v ammoniumformate buffer(0.05M,pH4)/MeOH, flow 1 ml/min).

Typical chromatograms of (ultrafiltered) uremic serum and an uremic serum PBA-extract are shown in FIG.1.

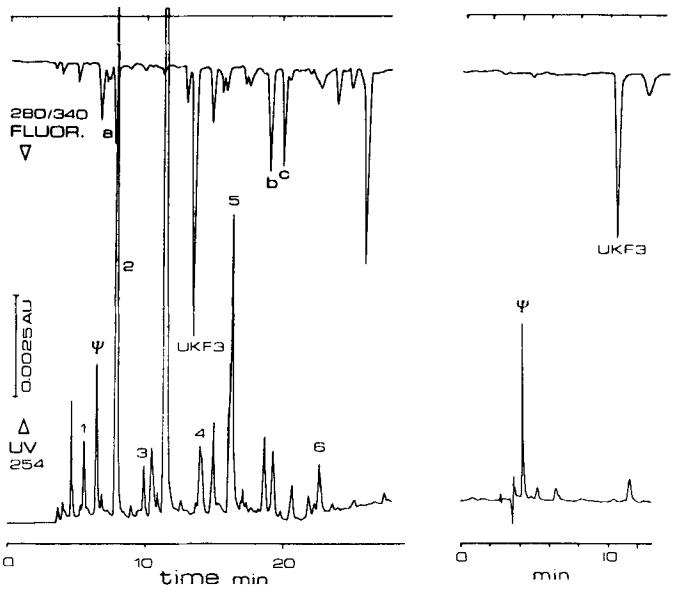

FIG.1. HPLC chromatograms of ultrafiltered uremic serum (left, gradient elution, conditions see [2,3]), and of an uremic serum PBA-extract (right, isocratic, conditions see text). Equipment for both gradient and isocratic analyses as in [2,3]. 1,creatinine; ψ,pseudouridine; 2,uric acid; 3,hypoxanthine; 4,furoylglycine(tent.); 5,*p*-hydroxyhippuric acid; 6,hippuric acid; a,tyrosine; b,indoxylsulfate; c,tryptophan [4].

TABLE I. Recoveries of the PBA-extraction (mean (S.D.)), n=6.

Analyte	unbuffered serum	buffered serum	buffered aqueous solution (PSI)
PSI	76.7 (4.9)	88.3 (3.3)	97.3 (5.0)
UKF3	96.9 (2.2)	97.8 (3.5)	

Conclusion. Pseudouridine can be analyzed rapidly and reliably by isocratic HPLC after sample pretreatment by PBA solid-phase extraction. Approximately 25 samples can be analyzed daily free from interference by the numerous solutes accumulating in uremic serum. The unknown fluorescent solute "UKF3" probably contains a *cis*-diol group, which can be concluded from the high recovery on *cis*-diol specific PBA extraction.

References.

[1] Asatoor AM. *Clin Chim Acta* 1968;20:407-411. **[2]** Schoots AC, Gerlag PGG, Mulder AW, Peeters JAG, Cramers CAMG. *Clin Chem* 1988;34:91-97. **[3]** Schoots AC, Dijkstra JB, Ringoir SMG, Vanholder R, Cramers CAMG. *Clin Chem* 1988;34:1022-1029. **[4]** Schoots AC, Dissertation, Eindhoven University of Technology, 1988, The Netherlands.